

# BIODEGRADATION OF TEXTILE DYES BASED ON WOOD CARRIERS

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## ABSTRACT

Biodegradation of textile dyes from the azo class by *Bjerkandera sp.* and microbial consortium from forest residues. *Bjerkandera's* degradation ability was evaluated in unsterile environment and put out of competition from the microbial consortium inhabiting forest residues. Microorganisms from forest residues were evaluated and transferred to soft wood shavings with maintained biodegrade performance. A continuous system was created containing anaerobic and aerobic parts and a successful degradation was monitored by spectrophotometer and High pressure liquid chromatography (HPLC).

## KEYWORDS

Continuous system, lignocellulosic material, HPLC, textile waste water treatment.

## 1 INTRODUCTION

Textile fibres are all constructed by polymeric organic fibres, which can be synthetic, semi-synthetic or natural fibre. Because of these different fibre types different dyes and processes have evolved to colour the material.

One of the most used dyes today is reactive azo dyes, which react chemically to the fibre and form a covalent bond between the dye molecule and the fibre. The dye molecule can also react with the alkaline ions in the solution; the hydrolysed dye which is formed is no longer capable of reacting with the fibre and so it must be washed out before dyeing is complete. [4] The drawback with reactive dyes is that 10-40 % of the dyes never react with the fibre and the need for a wash-off step in the process. [5] Dye contents in waters treated in studies performed differs quite a bit, in the artificial waters from 20 mg l<sup>-1</sup> to 2.5 g l<sup>-1</sup>, while normally 10-50 mg l<sup>-1</sup> is found in dye house effluents [11]. O'Neill [11] also states that a typical dye effluent from a textile mill contains several dyes.

Today the water demands and chemicals used in the process of forming textile fabrics is a rising environmental issue. In 2000 the EU production of fabrics represented 3.4 % of the manufacturing industry's turnover and 6.9 % of the industrial employment [5].

There are several methods to treat textile waste water. Treatment with different filters is very attractive since they open the opportunity to reuse water. The filter performance depends on parameters such as pH and concentrations. Filtration is divided into three categories: Ultrafiltration, nanofiltration and reverse osmosis. The membranes are constructed from several different polymer materials [6, 7]. The drawback is that filter techniques demand high investment and operational cost.

Dyes can also be degraded with oxidative processes that either use UV (Photodegradation) or combined with a catalyst and hydrogenperoxide ( $H_2O_2$ ) or ozone ( $O_3$ ). Different catalysts are used as Co-ZSM-5,  $Fe_2O_3$ , CdS, ZnO,  $WO_3$ , but most preferred is  $TiO_2$  [3]. Similar mechanism is also performed by electrochemical degradation methods, most well established is the Fenton process. However these methods demands investments and operational costs. Biological processes can be performed by different living organisms; in sewage treatment bacteria and microscopic animal play important roles. Scientists from Environmental Protection Agency's (EPA's) Water Engineering Research Laboratory in Cincinnati, Ohio, studied the fate of water-soluble acid and direct dyes in the activated sludge process. 11 out of 18 textile dyes passed through the pilot-scale, activated sludge process untreated, 4 adsorbed onto the activated sludge and 3 underwent biodegradation.[12]

Anaerobic bacterial processes have proven able to degrade azo dyes, this probably due to the electrochemical unstable azo bond ( $-N=N-$ ), which can act as an electron receiver. The enzyme Azoreductase is used to donate electrons to the substrate (electron acceptor) [2, 16]. However oxygen is a better electron acceptor and the azo bonds potential to receive electrons is reduced in the presence of oxygen [14, 16] Several bacteria have proven degradation activity and ability to produce the enzyme azoreductase. Investigations on intestinal bacteria have proven that species like *Clostridium*, *Enterococcus* and *Bifidobacterium* was able to split the azo dye direct blue 15 during anaerobic conditions. Alex [1] worked with a microbial consortium isolated from effluent water from Urafiki Textile factory and found that 0.3 % glucose or 0.2 % yeast extract to give the best enhancement in decolourization. Carbon can also be attained from lignocellulosic carrier material and if dye molecules are degraded aromatic amines become available. The azo dyes can give rise metabolites, often aromatic amines, some of them even more toxic than the dye they origin from. [13] To decolourize water is just one part of the purification process; the next step is to mineralise the aromatic amines formed. Aromatic amines are smaller molecules which should be able to enter the bacterial cell to be mineralised on the inside. Several researchers have seen big decrease in toxicity from the effluent of the anaerobic step to effluent in the following aerobic step [10, 8]. Van der Zee and Villaverde [15] come in their review article to the conclusion that most aromatic amines are mineralised in the aerobic step.

Working with the aim to provide textile waste water treatment technology in developing countries it is important to keep operational and investment costs to a minimum.

## 2 METHOD

### 2.1 Dyes

### 2.2

Two of the most common azo dyes were used in this study, Reactive Red 2 and Reactive Black 5, molecular structure in figure 1.

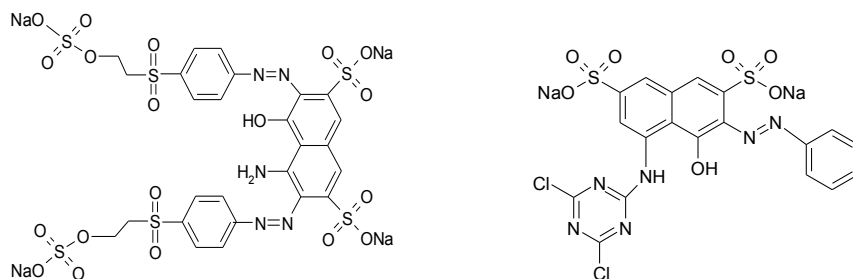


Figure 1: Molecule structure of Reactive Black 5 (left) and Reactive Red 2 (right)

### 2.3 Fungus

The first experiment was designed to use the White-root fungi *Bjerkandera sp.* Strain BOL-13 gene bank accession nr AY633927, which previous performed good degradation on both artificial waste water and real waste water from Urafiki textile industry in Dar es Salaam, Tanzania [9]. The fungus was inoculated on malt agar medium (48g l<sup>-1</sup>) in petri-dishes and incubated at room temperature. The media was autoclaved 15 min at 121° C prior to inoculation. In order to be cost efficient and sustainable, chips of forest residues were chosen as carrier to support growth of the fungus. The wood chips were inoculated by a 1 cm<sup>2</sup> piece of malt agar overgrown with mycelia. The fungus was allowed to grow until it had been established on the wood chips. *Bjerkandera* was cultivated in unsterile environment and decolourization ability of 200 mg l<sup>-1</sup> Reactive Red 2 200 mg l<sup>-1</sup> Reactive Black 5 with 1g l<sup>-1</sup> of yeast extract was examined. Each experiment consisted of three replicates, with wood chips from forest residues as a carrier and substrate for the microorganisms. The experiments were designed with the composition shown in table 1. The flasks had the small plastic net installed in the middle of the 500 ml E-flasks, see figure 4. In each flask 70 g of moisture forest residues wood chips, collected three days earlier from VEAB in Växjö, were put at one side of the net. To get the wood chips sterile, they were autoclaved 1 × 99 minutes at 135 ° C three consecutive days. The net and the flask are sterilised and all the pieces are put together in a sterile bench. The flasks were filled with 300 ml of liquid, sterile water for the controls and sterile dye mix for the others, booth autoclaved for 15 minutes in the autoclave. Series 1<sub>A, B & C</sub> and 2<sub>A, B & C</sub> were inoculated by 1 cm<sup>3</sup> of agar overgrown with mycelium of *Bjerkandera sp.*

Table 1: *Bach exp. 3 composition of carrier, micro organisms and artificial waste water.*

Batch	Type of chips	Sterilised	<i>Bjerkandera</i>	Dye type 200 mg l <sup>-1</sup> of each dye	Yeast extract (1g l <sup>-1</sup> )	Amount of liq. (ml)
control	Forest residues	Yes	No	-	-	300
1A	Forest residues	Yes	Yes	Red + black	Yes	300
1B	Forest residues	Yes	Yes	Red + black	Yes	300
1C	Forest residues	Yes	Yes	Red + black	Yes	300
2A	Forest residues	No	Yes	Red + black	Yes	300
2B	Forest residues	No	Yes	Red + black	Yes	300
2C	Forest residues	No	Yes	Red + black	Yes	300
3A	Forest residues	No	No	Red + black	Yes	300
3B	Forest residues	No	No	Red + black	Yes	300
3C	Forest residues	No	No	Red + black	Yes	300

## 2.4 Transfer of microbial consortium

To improve the analytical analysis the microflora from forest residues were transferred to soft wood shavings, three different ways of inoculation were tested. 1) The microflora were transferred by inoculate with three wood chips of forest residues without bark, which were chosen from three different places in the sack. 2) Approximately 100g of forest residues wood chips in water with stirring during ½ h and then the water was separated by decantation, and had a dark brown colour from all the particles in it. 3) Batch H8 had wash water filtrated through filter munktell nr 3, for experimental overview se table 2.

Table 2: Series H<sub>5,9</sub>: Composition of carrier, micro organisms and artificial waste water

Batch	Wet weight of shavings (g)	Sterilised	Treatment/inoculation	Dye type 200 mg l <sup>-1</sup> of each dye	Yeast extract (1g l <sup>-1</sup> )	Amount of liq. (ml)
H5	39,1	Autoclaved: 15 min, 220 °C	-	Red + black	No	350
H6	41,8	No	3 forest residues chips	Red + black	No	250
H7	40,7	No	20 ml forest residues wash water	Red + black	No	250
H8	43,4	No	20 ml forest residues wash water, filtrated	Red + black	No	250
H9	42,8	Autoclaved: 99 min, 135 °C	-	No	No	250

## 2.5 Continuous system

With the results from these studies above and literature review the continuous system in figure 2 was constructed. The continuous system was designed with two anaerobic reservoirs with a volume of 1 l each, filled with approximately 100 g of soft wood shavings each (moist cont. 78-80 %). The third reactor was aerobic and had a volume of 500 ml and 28 g of soft wood shavings (moist cont. 80 %). This reactor was aerated with compressed air through an air stone in the bottom.

The system was feed by artificial waste water composed of 200 mg l<sup>-1</sup> Reactive Red 2 200 mg l<sup>-1</sup> Reactive Black 5 with 1g l<sup>-1</sup> of yeast extract.

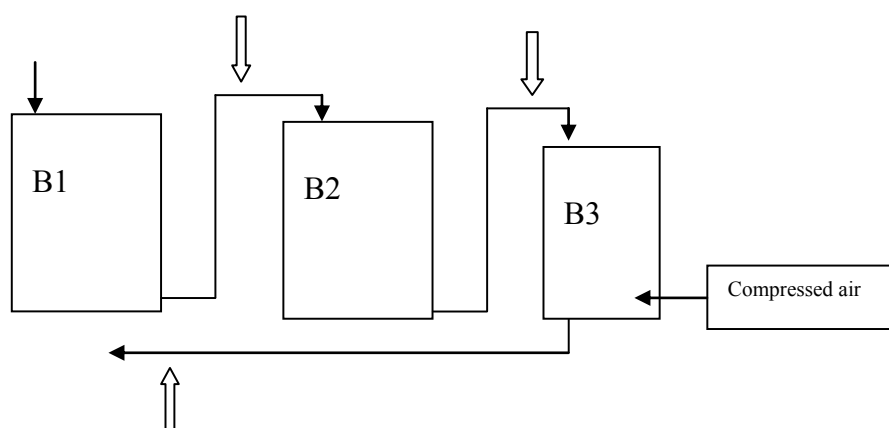


Figure 2: Continuous system

## 2.5 Analysis

Samples were filtered through a 0.45 µm filter (Sartorius minisart). The samples were scanned in a spectrophotometer, Perkin Elmer lambda 35 UV/VIS and analysed by Perkin

Elmer UV winlab ver: 2.85.04 software, from 190 nm to 750 nm. The samples from the experiments were diluted 1:10 by taking 100µl sample, add 900 µl of deionised water.

Some samples were analysed using the HP 1100 High pressure liquid chromatography (HPLC) system (Hewlett Packard, Waldbronn, Germany). The HPLC system was equipped with a G1314A deuterium lamp detector and a Fortis C18 250 x 4.6mm, 3 µm column (Dalco Chromtech, Sollentuna, Sweden), protected by a guard column containing the same packing material. The software used for processing and reporting data was ChemStation B.02.01 SR2 (Agilent Technologies, Santa Clara, CA, USA). The mobile phase was a gradient starting with 80% 50 mM formic acid / 3.03 mM Ammoniumformiat buffer (pH 2.8) (A) and 20% methanol (B). The gradient increased linearly to 100% (B) and 0% (A) over 45 min, and this condition was maintained for 5 min; the post phase continued at 20% (B) and 80% (A) for 10 min. The detection was performed at 280 nm.

### 3 RESULTS AND DISCUSSION

#### 3.1 Fungus

An evaluation of the results from day 19; line 1 (sterile with *Bjerkandera*) performed 53 %, line 2 (unsterile with *Bjerkandera*) 59,6 % and line 3 (unsterile) 70,1 % decolourization. The fungus *Bjerkandera* did not perform as well as expected, but the microflora from forest residues proved interesting degradation ability. Wood has an interesting composition of cellulose, hemicellulose, pectin, lignin and resins that should support and evolve an interesting microflora with capacity to degrade variable molecular structures.

The experiment showed good decolourization visually and by spectrophotometer the first 20 days, but after day 23 absorbency increased again, probably due to substances released from the forest residues used as a supporting material (figure 4).

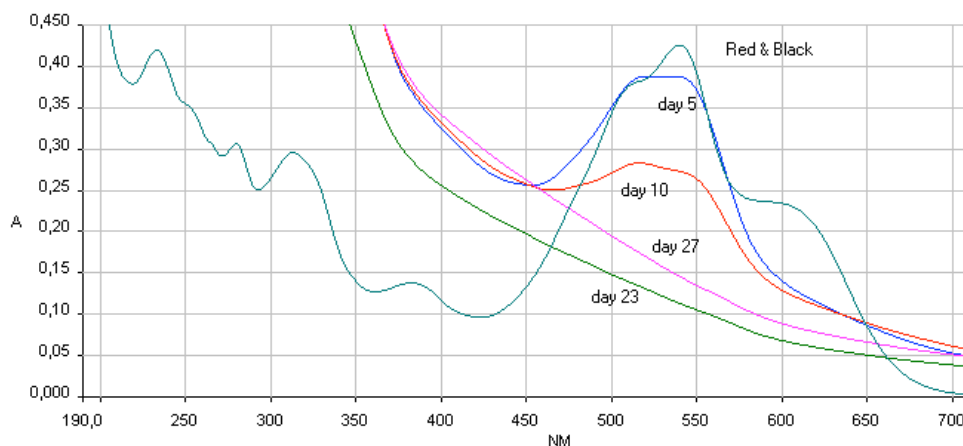


Figure 4: Absorbance series 3B unsterile *F. res. chips*, *Bjerkandera sp.* and yeast extract.

#### 3.2 Transfer of microbial consortium

The three different way of inoculation seemed to all work well, with the best results for unfiltered rinse solution. After 16 days the decolourization was for H6 75.4 %, H7 81.7 %, H8 71.8 % at 550 nm. Figure 5 show a visual example of the degradation in the H-series. The degradation continued further and after 43 days over 90 % of the colour has disappeared.

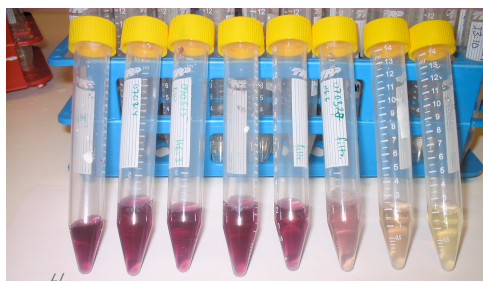
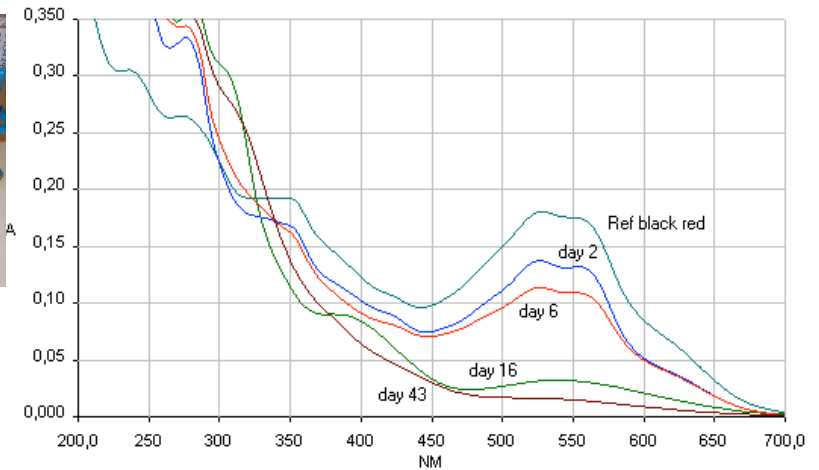


Figure 5: visual result of degradation H-series (H7), day 1-43.



### 3.3 Continuous system

The continuous system was in use for 45 days, with a turnover rate at approximately 4 days and 20 hours each for the large reservoirs and 4 days 9 hour for reservoir three. The system had a total turnover rate of 14 days and 1 hour.

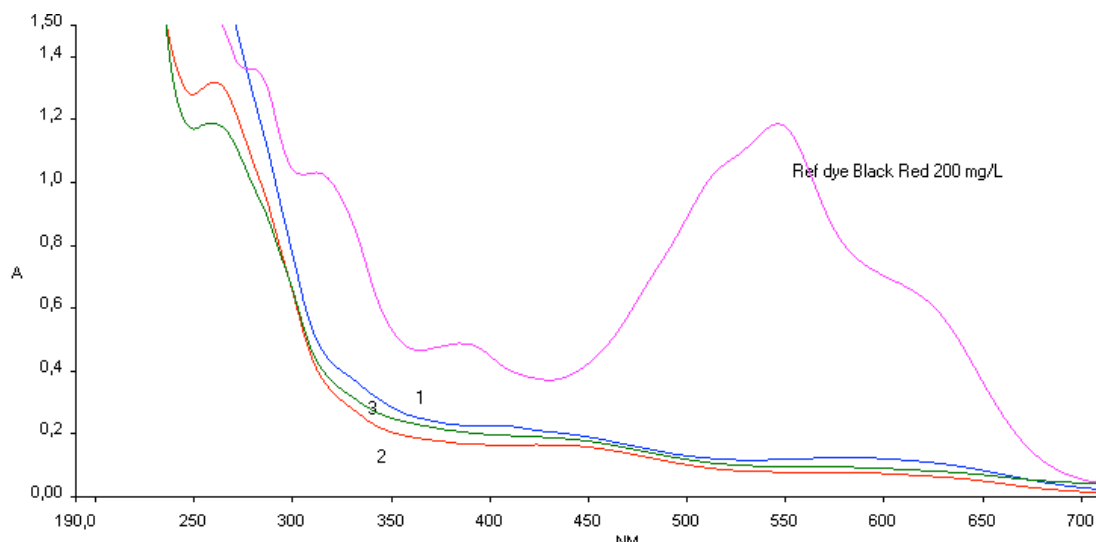


Figure 6: degradation in continuous system.

The graph in figure 6 shows clearly that the colour disappears in the first reservoir, probably due to breaking of azo bond. The decolourization at 550 nm was for reservoir 1 89.8 %, reservoir 2 93.3 % and reservoir 3 91.9 %. However the fate of the metabolites is unclear.

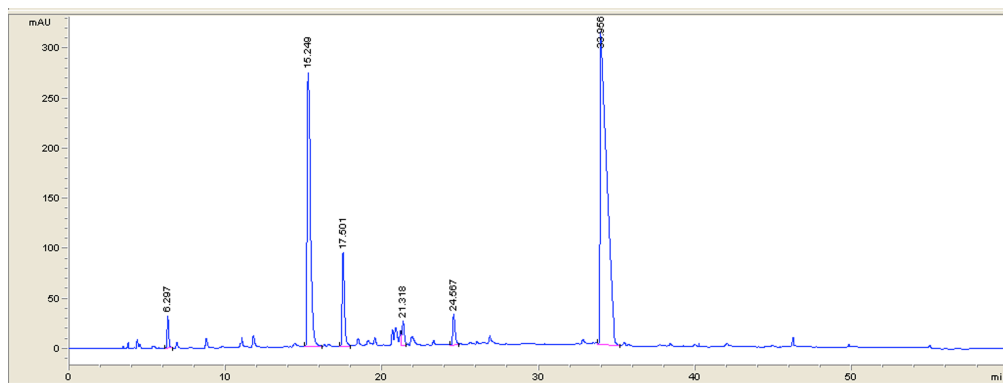
The HPLC show that the dye molecules are degraded in reservoir 1, but also display metabolites, which was reduced in reservoir 2 and 3. Figure 7 a display the profiles for the two azo dyes, with major peaks at retention time (rt) 15.25 and 33.96. The HPLC diagram from outlet of reservoir 1 show no presence of the dyes profile, but a major peak at rt 3.79 and several small peaks from rt 4-22. In reservoir 2 (figure 7c) the peak at rt 3.79 and several of the minor peaks from rt 7.6-22 have been reduced. From the third reservoir with aerobic environment, an increase of the peaks at rt 3.79 and 14.75 and reduction of peaks at rt 4.2-6.8 is displayed. The continuous system efficient decolourized the artificial wastewater on merely 4 days (reservoir 1), the metabolites were tougher to metabolise, but was reduced by the

further treatment in anaerobic and aerobic environment. Van der Zee and Villaverde [15] state in their article that an aerobic step after anaerobic degradation of azo dyes decrease the toxicity in the water, most aromatic amines are mineralised.

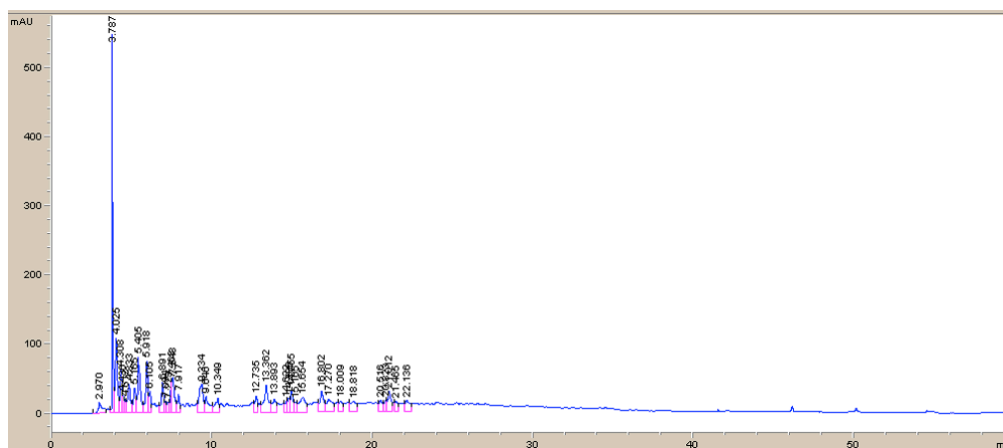
A system like this should be possible to implement at the ponds present at most textile mills today, especially since normal dye concentration in textile waste water is 10-50 mg l<sup>-1</sup> [11].



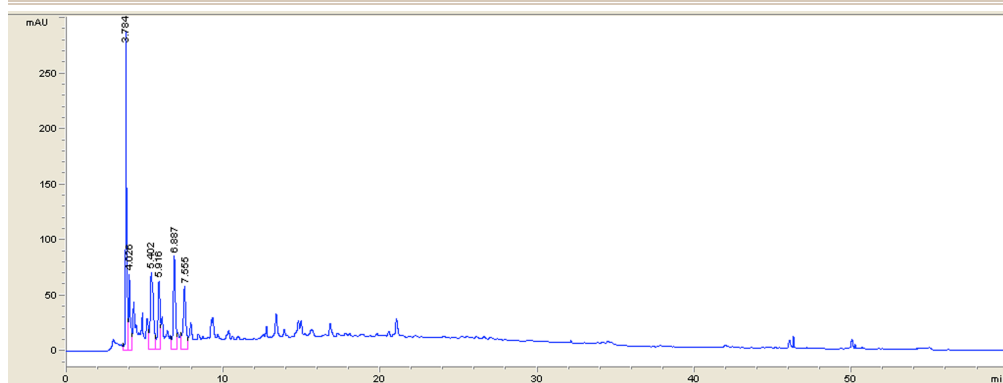
7a



7b



7c



7d

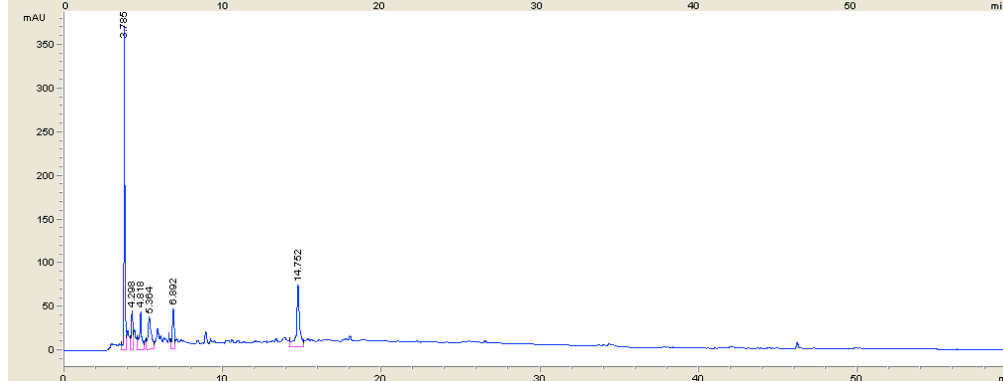


Figure 7: a) Reference dye Reactive Black 5 and Reactive Red 2, b) HPLC reservoir 1 080528, c) HPLC reservoir 2 080528, d) HPLC reservoir 3 080528

## 4 CONCLUSIONS

The fungus *Bjerkandera* strain BOL 13 was able to grow on sterile forest residues but had trouble competing with the microbial community inhabiting the forest residues. The microbial community on the other hand performed even better decolouration of the azo dyes used.

The transfer of microorganisms from forest residues was successful, and a community able to decolourize the azo dyes Reactive Black 5 and Reactive Red 2 was able to establish on the soft wood shavings.

A continuous system based on wood shavings and a consortium of microorganisms from forest residues is able to decolourize high concentrations of the two azo dyes Reactive Red 2 and Reactive Black 5 and mineralize most of the metabolites.

## 5 ACKNOWLEDGEMENTS

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